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Retention of KOH-soluble fluoride formed after application of a SnCl(2)/AmF/NaF containing mouth rinse under erosive conditions

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Abstract: **OBJECTIVE:** Application of SnCl(2)/AmF/NaF containing mouth rinse showed good protection against erosion. The aim of the study was to evaluate if this is due to the amount of KOH-soluble fluoride (KOHsF) formed or its resistance under erosive conditions. **METHODS:** One hundred and fifty bovine enamel samples were allocated to five groups (n = 30) and were once eroded in 0.05 mol/l citric acid (5 min). Samples were stored in artificial saliva for 4 days. Samples of two groups (erosive-SnCl(2) + erosive-NaF) were eroded 6 × for 5 min. The remaining samples were stored in aqua dest deionised water. Each day the samples were treated twice for 2 min with 1 ml SnCl(2)/AmF/NaF-solution (erosive-SnCl(2);neutral-SnCl(2)/AmF/NaF) or NaF-solution (erosive-NaF;neutral-NaF). The fifth group remained untreated (control). On day 5, 10 samples of each group were used for determination of KOHsF (series 1). The remaining samples were again eroded (erosive-SnCl(2) + erosive-NaF) or stored in artificial saliva (neutral-SnCl(2) + neutral-NaF). KOHsF of another 10 samples of each group was measured (series 2). The last 10 samples of each group were also treated as described above and the amount of KOHsF was measured (series 3). **RESULTS:** In each series 1-3 KOHsF in group erosive-SnCl(2)/AmF/NaF were significantly higher. No significant loss of KOHsF between the series 1-3 was observed (except for control). **CONCLUSION:** SnCl(2)/AmF/NaF containing mouth rinse revealed a better formation of KOH-soluble fluoride as the NaF-solution, although the applied fluoride compound has no influence on the stability of the KOHsF under erosive conditions, leading to the conclusion that the resistance of KOHsF is not responsible for the difference in the protection against dental erosion.

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Retention of KOH-soluble fluoride formed after application of a SnCl₂/AmF/NaF containing mouth rinse under erosive conditions.

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Abstract:

Objective: Application of $\text{SnCl}_2/\text{AmF}/\text{NaF}$ containing mouth rinse showed good protection against erosion. Aim of the study was to evaluate if this is due to the amount of KOH-soluble fluoride (KOHsF) formed or its resistance under erosive conditions.

Methods: 150 bovine enamel samples were allocated to five groups ($n=30$) and were once eroded in 0.05 mol/l citric acid (5 min). Samples were stored in artificial saliva for four days. Samples of two groups (erosive- SnCl_2 +erosive-NaF) were eroded 6x for 5 min. Remaining samples were stored in aqua. dest. Each day the samples were treated twice for 2 min with 1 ml $\text{SnCl}_2/\text{AmF}/\text{NaF}$ -solution (erosive- SnCl_2 ;neutral- $\text{SnCl}_2/\text{AmF}/\text{NaF}$) or NaF-solution (erosive-NaF;neutral-NaF). Fifth group remained untreated (control). On day 5, 10 samples of each group were used for determination of KOHsF (series 1). Remaining samples were again eroded (erosive- SnCl_2 +erosive-NaF) or stored in artificial saliva (neutral- SnCl_2 +neutral-NaF). KOHsF of another 10 samples of each group was measured (series 2). Last 10 samples of each group were also treated as described above and the amount of KOHsF was measured (series 3).

Results: In each series 1-3 KOHsF in group erosive- $\text{SnCl}_2/\text{AmF}/\text{NaF}$ were significantly highest. No significant loss of KOHsF between the series 1–3 was observed (except for control).

Conclusion: $\text{SnCl}_2/\text{AmF}/\text{NaF}$ containing mouth rinse revealed a better formation of KOH-soluble fluoride as the NaF-solution, although the applied fluoride compound has no influence on the stability of the KOHsF under erosive conditions, leading to the conclusion that the resistance of KOHsF is not responsible for the difference in the protection against dental erosion.

Key words:

$\text{SnCl}_2/\text{AmF}/\text{NaF}$; KOH-soluble fluoride; erosion

Introduction:

The uses of fluorides in different forms and different application modes have caused a reduction in the prevalence of dental hard tissue loss due to caries over the last decades [1-4]. Due to this decline of dental hard tissue loss due to caries, other reasons for dental hard tissue loss have entered into the focus of dental research, namely erosion [5]. Erosive tooth wear is associated with contact of dental hard tissues with acids or chelators in the absence of microorganisms [6]. Sources of extrinsic acids are mostly dietary caused like acidic foodstuff and beverages [7]. The results of those acid attacks are the dissolution of the tooth surface, which may occur on a relatively rapid time scale [8].

The presence of fluoride ions in a solution may decrease demineralisation by reducing the solubility of the tooth surface [9]. The frequent use of different, topically applied, fluoride preparations is recommended to prevent erosive tooth wear [9].

Beside this traditional preventive approaches using fluoride compounds such as sodium fluoride, amine fluoride or sodiummonofluorophosphate to prevent caries and erosion caused tooth wear, different complexes of metal cations with or without fluoride have been used to establish new and more effective formulations to prevent erosive tooth wear [10]. Studies treating enamel with stannous ions combined with fluoride have shown promising results in reduction of mineral loss under erosive conditions [10-12].

The application of topically applied fluorides leads to a formation of a calcium fluoride like precipitate [13]. During further erosions, the demineralisation of dental hard tissues is inhibited in the presence of this calcium fluoride like precipitate [14].

Taking in consideration this assumption, the retention and the amount of the calcium fluoride like precipitate is important for the prevention of possible

prospective dental hard tissue loss due to erosion [14,15].

Therefore, aim of this in vitro study was to evaluate in how far a calcium fluoride like precipitate would be formed due to the application of a commercial available mouth rinse containing stannous ions combined with fluoride and how resistant this calcium fluoride like precipitate is under erosive conditions. It is suggested that the better protective effect against dental erosion of the mouth rinse containing stannous ions combined with fluoride [12] is related to the formation of higher and less susceptible amounts of calcium fluoride like precipitate. The hypothesis of the present study was, that the calcium fluoride like precipitate, formed after the application of the stannous ions containing fluoride mouth rinse, is less susceptible under erosive conditions compared with the calcium fluoride like layer formed after the application of a fluoride mouth rinse not containing stannous ions.

Materials and methods:

Sample preparation:

Thirty freshly extracted bovine lower incisors were used in this study. Age of cattles was between 2 1/2 and 3 years. For sample preparation, cores (3 mm in diameter) were drilled out of the buccal surface of the crowns with a trephine mill (per tooth five samples “A–E” were prepared). Samples were marked to ensure that the five samples from one tooth could be identified as such after further preparation. The enamel drilling cores were embedded in an acrylic resin (Palavit G, Kulzer Wehrheim, Germany) using steel moulds with an inner diameter of 6 mm. After removing the samples from the moulds, the enamel surface was ground with water-cooled carborundum discs (800, 1000, 1200, 2400 and 4000 grit; Water Proof Silicon Carbide Paper, Struers, Erkrath, Germany). Grinding was performed in an automatic grinding machine with running tap water as coolant. About 300 µm thickness of the

outermost enamel layer was removed. This loss was controlled with a micrometer device (Mitutoyo, Tokyo, Japan).

In total, 150 samples were prepared which were later allocated to five groups: all samples A to group “erosive-SnCl₂”, all samples B to group “erosive-NaF”, all samples C to “neutral-SnCl₂”, all samples D to group “neutral-NaF” and all samples E to group “control”.

Study design and allocation

All samples of all groups were eroded by immersing in 0.05 mol/l citric acid for 5 min before application of the respective solutions. For the erosion, the samples were stored in Eppendorf Tube (Eppendorf International, Hamburg, Germany) with 2 ml of 0.05 mol/l citric acid (pH 2.3). The erosion was performed under gentle agitation in a shaking water bath (LAUDA A120S, Lauda-Königshofen, Germany) at room temperature. Table with solutions used and group allocation is given in Table 1.

Then all samples were treated for four days in a demineralisation/ remineralisation cycle.

Per day the following procedure (precycling) was performed:

Each sample of two groups was treated with 1 ml of a SnCl₂/AmF/NaF-solution (elmex® erosion protection dental rinse, Gaba International AG, Therwil, Switzerland) for 2 min (groups erosive-SnCl₂ and neutral-SnCl₂). The samples of further two groups were treated with a sodium fluoride solution (Gaba International AG, Therwil, Switzerland) (groups erosive-NaF and neutral-NaF). The concentration of fluoride was 500 ppm for both solution and the pH was 4.5. Samples of the fifth group were treated with distilled water and were used to determine the baseline content of KOH-soluble fluoride of the respective teeth (control).

After the application of the respective solutions, all samples of all groups were subjected to the following demineralisation/remineralisation cycling. The samples of groups erosive-SnCl₂ and erosive-NaF were eroded in 0.05 mol/l citric acid for 5 min (2 ml/sample) as described above. The samples of groups neutral-SnCl₂, neutral-NaF and control were stored in aqua dest. during the same period of time. After this erosive challenge, the samples of all groups were again stored in artificial saliva for 60 min. This demineralisation/remineralisation cycling was performed six times per day. After the final storage in artificial saliva for 60 min of the day, the respective solutions were applied again for 2 min. Overnight the samples were stored in artificial saliva (100 ml/10 samples of the respective group).

The artificial saliva was prepared following the composition given by Klimek et al. (1982) [16]. The artificial saliva was kept under constant motion during the whole experimental procedure.

On the fifth day, the testing of the retention of KOH-soluble fluoride was performed:

All samples of teeth 1–10 (series 1) of all groups were then used for the determination of KOH-soluble fluoride uptake (first measurement, baseline). The samples of the remaining teeth 11–30 were used in the following demineralisation protocol, prior to the determination of the KOH-soluble fluoride. The samples of groups erosive-SnCl₂ and erosive-NaF were eroded in 0.05 mol/l citric acid for 5 min. The samples of the remaining groups (neutral-SnCl₂, neutral-NaF and control) were stored in aqua dest. during the same period of time. Then all samples of teeth 11-20 (series 2) of all groups were subjected to the second KOH-soluble fluoride measurement. The samples erosive-SnCl₂ and erosive-NaF of the remaining teeth 21 – 30 were once again eroded in 0.05 mol/l citric acid for 5 min. The samples of groups neutral-SnCl₂, neutral-NaF and control were again stored in aqua dest.

during the same period of time. In these samples (teeth 21–30, series 3) KOH-soluble fluoride was also measured. A flowchart of the whole experimental procedure is given in diagram 1.

Determination of KOH-soluble fluoride:

The amount of KOH-soluble fluoride on samples was analysed following the method described by Caslavská et al. (1975) [17]. Each sample was stored in an Eppendorf Tube (Eppendorf International, Hamburg, Germany) with 1 mL 1mol/L KOH for 24 h under constant motion. The KOH solution was neutralized with 1 mL 1M HNO₃ and buffered with 1 mL of TISAB II (Orion Research, Cambridge, USA). The fluoride content in the solution was measured with a fluoride sensitive electrode.

Statistical analysis:

Descriptive statistics (means, standard deviations) together with the corresponding 95% confidence intervals (95%CI) for each group and series were computed.

Due to the design of the study the observations within one group but between series were independent. Consequently, one-way ANOVA followed by Scheffé post hoc test were performed to compare the amount of KOH-soluble fluoride between different series (1,2,3) within one group.

Owing to the study design the observations within one particular series but between different groups are dependent because the samples stemmed from the same tooth. Repeated measures ANOVA was applied to detect differences in the amount of KOH-soluble fluoride between different groups (“erosive-SnCl₂”, “erosive-NaF”, “neutral-SnCl₂”, “neutral-NaF” and “control”) within the same series. The 95%CI of mean differences of paired comparisons between the groups was provided in order to

visualize the relevance of the effects.

Data were coded in EXCEL and analyzed with StatView Version 5.0.1 (SAS Institute Inc., Cary, N.C., USA) and SPSS Version 16. The results of the analyses with p-values lower than 0.05 were considered as statistically significant.

Results:

The amounts of KOH-soluble fluoride for each group and series are given in table 2.

Comparisons within the groups:

In group erosive-SnCl₂, the amount of KOH soluble fluoride in series 1 ($2.88 \pm 0.31 \mu\text{g}/\text{cm}^2$) was significantly lower compared with the amount in series 2 ($4.35 \pm 0.77 \mu\text{g}/\text{cm}^2$) and 3 ($4.92 \pm 0.93 \mu\text{g}/\text{cm}^2$) ($p = 0.0005$ and <0.0001 , respectively). KOH-soluble fluoride in series 2 and 3 were not statistically significantly different from each other ($p = 0.22$).

Within group erosive-NaF, there was no significant difference ($p = 0.53$) in the amounts of KOH-soluble fluoride in series 2 ($2.20 \pm 0.30 \mu\text{g}/\text{cm}^2$) and 3 ($2.42 \pm 0.66 \mu\text{g}/\text{cm}^2$). However, as compared to the amount in series 1 ($1.47 \pm 0.25 \mu\text{g}/\text{cm}^2$) both series 2 and 3 showed significantly higher values of KOH-soluble fluoride ($p = 0.0041$ and 0.0002).

Within groups neutral-SnCl₂ and neutral-NaF the amounts in series 1 (1.62 ± 0.18 and $1.19 \pm 0.13 \mu\text{g}/\text{cm}^2$), 2 (1.77 ± 0.57 and $1.18 \pm 0.16 \mu\text{g}/\text{cm}^2$) and 3 (1.59 ± 0.35 and $1.08 \pm 0.20 \mu\text{g}/\text{cm}^2$) were not statistically significantly different from each other ($p > 0.05$, respectively).

In the control group the amount of KOH-soluble fluoride in series 1 ($0.96 \pm 0.27 \mu\text{g}/\text{cm}^2$) was not statistically significant different compared with the amount in series 2 ($0.83 \pm 0.12 \mu\text{g}/\text{cm}^2$; $p = 0.28$). Also, the amount in series 3 ($0.75 \pm 0.09 \mu\text{g}/\text{cm}^2$) was not different compared with the amount in series 2 ($p = 0.63$). The amount of measurement 1 was significantly higher compared with the KOH-soluble fluoride measured in series 3 ($p = 0.048$).

Comparisons within the series:

In all series the amount of KOH-soluble fluoride was significantly higher when the respective fluoride solution (SnCl₂/AmF/NaF-solution or NaF solution) was applied during an erosive precycling instead of a neutral precycling (erosive-SnCl₂ vs neutral-SnCl₂ and erosive-NaF vs neutral-NaF). Furthermore, within the same kind of precycling (erosive or neutral), the amount of KOH-soluble fluoride of the samples treated with the SnCl₂/AmF/NaF-solution was significantly higher compared with the samples treated with the NaF solution (erosive-SnCl₂ vs erosive-NaF and neutral-SnCl₂ vs neutral-NaF). The amount of KOH-soluble fluoride in the control group was significantly lower compared with the amounts of all other groups.

Discussion:

For the present study, samples have been prepared from bovine lower incisors. Reason for choosing bovine teeth was that large uniform quantities of bovine teeth can be easily obtained while they are still having almost the same composition and physical properties as human enamel [18-20].

In the present study, the amount of KOH-soluble fluoride was determined by the method described by Caslavská et al. [17] as this method has been used in numerous other studies [21,22] considering the uptake of fluoride in/on enamel, is well-suited for the purpose investigated in the present study and well-established in our laboratory [23,24].

For the present study, all samples were immersed for 5 min in 0.05 mol/l citric acid before the start of the whole experiment to form a demineralised surface like it is found after drinking an erosive beverage.

As the present study aimed to evaluate the amount of KOH-soluble fluoride

formed under cyclic demineralisation/remineralisation conditions with a frequent application of the respective fluoride containing products, four days with consecutive cyclic application of acid and immersion of the samples in artificial saliva were performed before measuring the amount of KOH-soluble fluoride. This kind of demineralisation/remineralisation cycling with similar duration of erosive challenge, remineralisation and fluoride application has also been used in previous study investigating the present stannous containing fluoride dental rinse [10].

Limitation of the present study might be that the whole experiment was only performed with a single acid, pH and concentration as well under constant temperature and constant agitation. Due to this reason, only a very limited effect of the erosion could be simulated. Already in 1962, Gray [25] demonstrated that the erosive demineralisation depends on the stirring speed (velocity of acid), the temperature and pH value. In vivo, more difference in the erosive effect might be expected, as the temperature of acid changes, the pH and type of acids might vary and also the mode of drinking (velocity of acid) might differ. The pH drop in the oral cavity due to the consumption of acidic beverages is heavily influenced by the way of drinking [26]. The highest pH drop was observed, when the beverage was hold in the mouth, with a less pronounced drop due to long-sipping. The least drop of intra oral pH was observed, when the beverage was gulped. Due to these reasons, the present findings might differ when other erosion parameters are used.

By the findings of the present study, the hypothesis, that the calcium fluoride like precipitate, formed after the application of the stannous ions containing fluoride mouth rinse, is less susceptible under erosive conditions compared with a fluoride mouth rinse not containing stannous ions, has to be rejected. No significant loss of KOH-soluble fluoride due to the erosive attack was observed either for the samples treated with the stannous ions containing fluoride mouth rinse or the samples treated

with the NaF containing mouth rinse. Reason for this finding might be, that the better protective effect against erosions of the stannous ions containing fluoride mouth rinse is not related to a less susceptible calcium fluoride-like precipitate but to an incorporation of the stannous ions in the enamel surface [12,27]. Furthermore, for the test groups with the erosive precycling the amount of KOH-soluble fluoride in the present study increased with the numbers of erosive attacks. It might be supposed that this finding indicates that the KOH-soluble fluoride on the samples with the erosive precycling is tightly bound and stable, so that it is not totally dissolved during the 24 h storage in KOH and can only be totally dissolved if it has been affected by an acidic solution before. To verify this suggestions further studies examining the surface after the storage in KOH has to be performed.

In contrast to the findings by Ganss et al. [22], no significant loss of KOH-soluble fluoride due to erosive or neutral attacks was observed in the present study. This discrepancy might be explained by the different storage periods between the consecutive measurements in the different studies. In the study by Ganss et al. (2007) the storage period, in which the samples were eroded and stored in remineralisations solution or only stored in remineralisations solution, was minimum 2 days while in the present study this interval was maximum 10 min (storage in aqua. dest or immersion in acid).

The amount of KOH-soluble fluoride was significantly higher if the respective fluoride compound was applied during the erosive precycling. This finding might be explained with the cyclic erosive attack on the enamel, resulting in an enlargement of the enamel surface while in the groups with the neutral precycling the enamel surface is only once erosively altered. This more enlarged enamel surface, in the erosive precycling groups, is more reactive and more KOH-soluble fluoride can be formed in the same time period of the fluoride application [28,29].

Within the same kind of precycling, the amounts of KOH-soluble fluoride formed during the application of the stannous ions containing fluoride mouth rinse were significantly higher compared with the amounts formed during the application of the NaF solution. As the calcium fluoride-like precipitate, which can be measured as KOH-soluble fluoride, is formed from calcium originating from the enamel and fluoride from the fluoride compound, the amount of calcium and fluoride ions, are the determination factors for the amount of calcium fluoride-like precipitate [30]. The two different fluoride compound had the same pH and the same concentration of fluoride, so the difference of formed amounts of KOH-soluble fluoride has to be attributed to the SnCl_2 . It might be imaginable, that the Sn can replace the Ca in the hydroxyapatite of the enamel due to its smaller atomic radius and its higher electric charge valence. The incorporation of Sn in the enamel has been proved in a recent study [12]. The replacement of Ca by Sn leads to a release of more calcium ions, ready to react with the fluoride of the fluoride solution and resulting in a higher amount of KOH-soluble fluoride. Taking in consideration that the stannous ions containing fluoride mouth rinse (SnCl_2 , AmF and NaF) contains more different ions than the pure NaF solution, it is furthermore imaginable that different kinds of fluoride precipitates might be formed on the enamel. It might be presumed that the higher amount of KOH-soluble fluoride after application of the stannous ions containing fluoride mouth rinse is related to this formation of different precipitates. Beside this more theoretical assumption, it has been shown in different other studies [31-34] that the uptake of fluorides from fluoride products is enhanced when amine fluoride is used as fluoride compound instead of sodium fluoride only. The difference in the amount of fluoride uptake from the two fluoride compounds (AmF and NaF) is attributed to the fact that the absorption of the amine molecule to the surface is pronounced and supports the adhesion and precipitation of the fluoride ion. As the here used stannous ions containing fluoride mouth rinse also contains amine fluoride, the higher amount of KOH-soluble fluoride

formed during the application of the stannous ions containing fluoride mouth rinse might also be related to these circumstances.

On one hand, the KOH-soluble fluoride formed after the application of the stannous ions containing fluoride mouth rinse showed the same susceptibility due to erosion than the KOH-soluble fluoride formed due to the application of a conventional sodium fluoride solution in the present study. On the other hand, the here used stannous ions containing fluoride mouth rinse has shown a highly protective effect against erosion in a previous study [35]. Taking in consideration these findings and that the cariostatic effect of fluorides is related to the KOH-soluble fluoride on the enamel surface [15] one might assume that the here used stannous ions containing fluoride mouth rinse will protect enamel against tooth wear not only due to erosion but also due to caries.

Conclusion:

By the findings of the present study it can be concluded that the stannous ions containing fluoride mouth rinse revealed a better formation of KOH-soluble fluoride as the NaF-solution although the kind of applied fluoride compound has no influence on the stability of the KOH-soluble fluoride under erosive conditions. Furthermore, due to these findings it can be concluded that not the resistance of the formed KOH-soluble fluoride might be responsible for the difference in protection against dental erosion found in previous studies [10-12,35].

As the calcium fluoride like precipitate plays an important role in the prevention of caries, it might be concluded, that the here used stannous ions containing fluoride mouth rinse will show the same caries preventive effect as the NaF-solution.

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Legends to tables and figures:

Tab. 1: Group allocation with the respective kind of precycling and the used solutions.

Dia. 1: Flow-chart of the experimental protocol

Tab. 2: Mean values (95% confidence interval) of KOH-soluble fluoride ($\mu\text{g}/\text{cm}^2$) for each group and series. Furthermore the applied solution and the kind of precycling are given.

Values within one group for the different series that are not statistically different are marked with same capital letters. All comparisons within one series of the groups with either the same kind of precycling (erosive- SnCl_2 vs erosive- NaF and neutral- SnCl_2 vs neutral- NaF) or the same kind of applied solution (erosive- SnCl_2 vs neutral- SnCl_2 and erosive- NaF vs neutral- NaF) or compared with the respective control showed statistically significant differences.

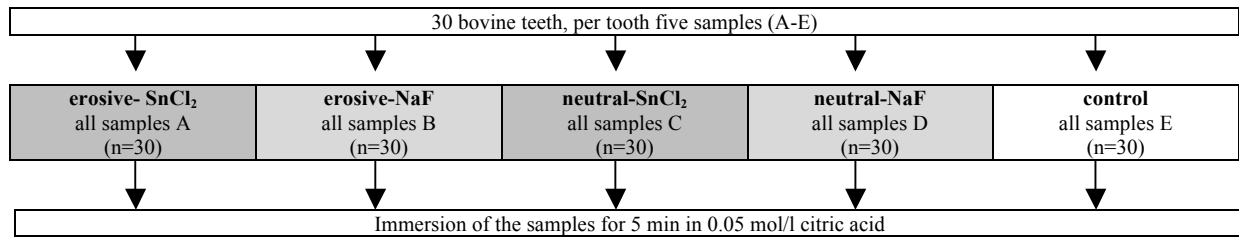
Tables:

	erosive- SnCl₂	erosive-NaF	neutral-SnCl₂	neutral-NaF	control
precycling	erosive	erosive	neutral	neutral	neutral
applied solution	SnCl ₂ /AmF/NaF (erosion protection)	NaF	SnCl ₂ /AmF/NaF (erosion protection)	NaF	-

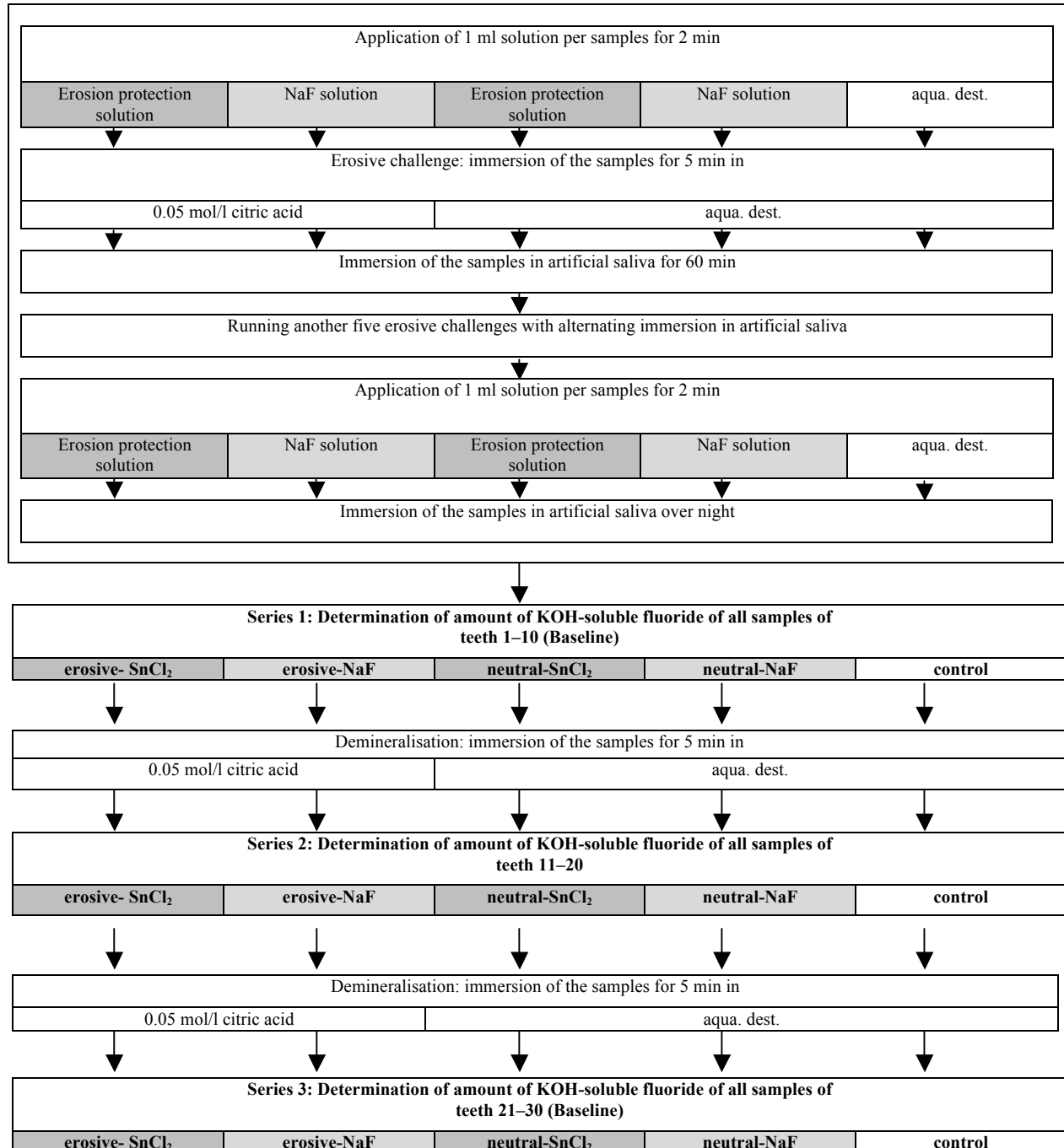
Tab. 1:

precycling	applied solution	group	series 1	series 2	series 3
erosive	SnCl ₂ /AmF/NaF	erosive- SnCl₂	2.88 A (2.67/3.10)	4.35 B (3.79/4.90)	4.92 B (4.25/5.59)
	NaF	erosive-NaF	1.48 A (1.30/1.65)	2.20 B (1.99/2.42)	2.43 B (1.96/2.90)
neutral	SnCl ₂ /AmF/NaF	neutral-SnCl₂	1.62 A (1.49/1.74)	1.77 A (1.36/2.18)	1.59 A (1.33/1.84)
	NaF	neutral-NaF	1.19 A (1.10/1.29)	1.18 A (1.07/1.29)	1.08 A (0.94/1.22)
untreated control		control	0.96 A (0.77/1.15)	0.83 A,B (0.74/0.91)	0.75 B (0.69/0.82)

Tab. 2:



The following day protocol was run for four days:



Dia. 1: Flow-chart of the experimental protocol